

Z₁₂I-PL-2 was constructed by replacing the HindIII-ClaI fragment from pZHWTx12-IL2-SEAP (Rivera et al 1996), in which expression of the SEAP reporter gene is driven by a basal promoter from a minimal IL-2 promoter (Rivera et al., 1996) downstream from 12 tandem copies of a ZFHD1 binding site, with the oligonucleotides encoding the following polylinker:

5'-AAGCTTGCCCTGCAGCGGGAATTCCACTAGTCGAGATCTCCATCGAT-3', (SEQ ID NO: 1)

B2 HindIII

PstI

EcoRI

SpeI

BglII

ClaI

In addition, the ClaI-BamHI fragment, which contains the SV40 early gene intron and polyadenylation signal, was replaced by a ClaI-BamHI fragment that contains the 3'UTR from the SV40 late gene (amplified from pCAT3-Basic [Promega]). This target vector therefore contains, 5' to 3', 12 tandem copies of a ZFHD1 binding site, the basal promoter from the human IL2 gene, a polylinker, and a 3'UTR from the SV40 late gene.

- On Pages 44-46, please replace the paragraphs at lines 10-36, 1-35, and 1-20 respectively with the following text:

Human angiotatin:

(See Cao et al., J. Clin. Invest., 101:1055-1063, March 1998)

Amplify amino acids 1 to 472 from human plasminogen (secretory signal, the pre-activation peptide and kringle 1-4 region) using primers:

5'- t AAGCTT gccgccacc ATG GAA CAT AAG GAA GTG - 3' (SEQ ID NO: 2)

HindIII

B3 5'- t ATCGAT tta TTA ATC TGG AAG CAG GAC AAC -3' (SEQ ID NO: 3)

ClaI

Digest PCR product with HindIII-ClaI and ligate the resulting fragment into the HindIII-ClaI sites of the polylinker of Z₁₂I-PL-2. Confirm cloning by DNA sequencing.

Human prolactin:

(See Clapp et al., Endocrinology 133:1292-1299, 1993)

The 16 kD fragment of human prolactin is cloned by amplifying amino acids 1 to 221 using primers:

5'- t AAGCTT gccgccacc ATG ATG AAA GGG TCC CTC CTG - 3' (SEQ ID NO: 4)

HindIII

5'- t ATCGAT tta TTA GCA GTT GTT GTT GTG GAT -3' (SEQ ID NO: 5)

ClaI

Clone HindIII-ClaI fragment into the polylinker of Z₁₂I-PL-2, as described for angiostatin.

Murine flk-1:

(see Lin et al. Cell Growth and Differentiation 9:49-58 1998)

Amplify amino acids 1 to 736 using primers:

5'- t GAATTC gccgccacc ATG GAG AGC AAG GCG CTG - 3' (SEQ ID NO: 6)

EcoRI

5'- t ATCGAT tta TTA CTG GCA GGT GTA GAG GCC -3' (SEQ ID NO: 7)

ClaI

Clone EcoRI-ClaI fragment into the polylinker of Z₁₂I-PL-2, as described above.

Human angiopoietin-2:

(See Maisonpierre et al., Science 277:55-60, July 4, 1997)

Amplify amino acids 1 to 497 using primers:

5'- t GAATTC gccgccacc ATG TGG CAG ATT GTT TTC - 3' (SEQ ID NO: 8)

EcoRI

5'- t ATCGAT tta TTA GAA ATC TGC TGG TCG -3' (SEQ ID NO: 9)

ClaI

Clone EcoRI-ClaI fragment into the polylinker of Z₁₂I-PL-2, as described above.

Human B-interferon:

Amplify amino acids 1 to 187 using primers:

5'- t GAATTC gccgccacc ATG ACC AAC AAG TGT CTC - 3' (SEQ ID NO: 10)

EcoRI

5'- t ATCGAT tta TCA GTT TCG GAG GTA ACC -3' (SEQ ID NO: 11)

ClaI

Clone EcoRI-ClaI fragment into the polylinker of Z₁₂I-PL-2 as described above.

Human endostatin:

(see Sasaki et al., EMBO J., 17:4249-4256, 1998)

B3
cab
Since human endostatin is a C-terminal fragment of collagen XVIII, in order to express it as a secreted protein, it must be fused to a signal sequence. For this purpose, the vector Z₁₂I-hGH-ss-XbaI was constructed by inserting a modified hGH cDNA between the EcoRI and ClaI sites of Z₁₂I-PL-2. The hGH cDNA was modified by mutagenesis to add an XbaI site immediately downstream of the secretion signal.

Amplify amino acids 1154 to 1337 from human collagen XVIII using primers:

5'- t TCTAGA CAC AGC CAC CGC GAC TTC -3' (SEQ ID NO: 12)

XbaI

5'- t ATCGAT tta CTA CTT GGA GGC AGT CAT -3' (SEQ ID NO: 13)

ClaI

Digest the PCR product with XbaI-ClaI, and ligate the resulting fragment into XbaI-ClaI-opened Z₁₂I-hGH-ss-XbaI. Perform DNA sequence to confirm cloning of the endostatin gene in frame with the secretory signal.

The replacement paragraphs presented above incorporate changes as indicated by the marked-up versions below.

This application is a continuation of and claims priority to U.S.S.N. 09/151,001, filed September 10, 1998, which claims the priority benefit of PCT US98/04525, filed March 9, 1998 3/9/98, and is a continuation-in-part of U.S.S.N. 08/813,771, filed March 7, 1997 3/7/97, now abandoned, which is a continuation-in-part of U.S.S.N. 08/400,643, filed March 8, 1995 3/8/95, now abandoned, and ~~of~~ claims the benefit of priority from provisional application 60/013,014, filed March 8, 1995 3/7/96. International Application PCT/US98/04525 was published under PCT Article 21(2) in English.

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